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13. ABSTRACT (Maximum 200 Words)

The goal of this project is to test the hypotheses that: 1) *Subjects with GWI have reduced NAA in the basal ganglia and pons, which are not accounted for by confounds such as PTSD, depression, and alcohol abuse.* 2) *Reduced NAA in basal ganglia and pons correlates with CNS signs and symptoms of GWI.*

Thus far, we have mailed out a total of 2319 recruitment letters describing our study. Dr. Weiner has appeared on several radio and TV programs in response to our press release. Over 488 subjects have contacted us expressing interest in this study. Study procedures include a medical evaluation, clinical assessments, neurocognitive testing, startle testing, MRI/MRSI, and the "Haley Questionnaire." To date, we have studied a total of 176 subjects, 150 of whom have data for analysis. Of this total, 39 met criteria for GWI, 54 are controls, and 57 have an intermediate classification. We currently have 10 subjects scheduled for the remainder of the month. Although some preliminary data analysis has been performed, the current sample size is much too small for formal data analysis.

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INTRODUCTION

The primary goal of this project is to test the *a priori* hypotheses that: 1) Subjects with Gulf War Illness (GWI) have reduced N-acetyl aspartate (NAA) in the basal ganglia and pons, which are not accounted for by confounds such as PTSD, depression, or alcohol abuse. 2) Reduced NAA in the basal ganglia and pons correlates with central nervous system signs and symptoms of GWI. This project proposes to replicate and extend previous findings of Haley et al. on 200 subjects with GWI and 200 Gulf War Veteran (GWV) controls drawn from Northern California and its surrounding regions. To date we have recruited 208 subjects, and enrolled 176 into the study. Of these 155 have met inclusion criteria. In addition, 10 subjects have already been scheduled through the end of August. Currently, three subjects are being scheduled per week in an effort to catch up on recruitment lags from the first 2 years of the study. In April, the Department of Defense provided contact information for over 33,000 Gulf War veterans residing in California. We have been mailing out 150 recruitment letters per week, which has yielded 40 calls from potential participants per month. We expect to continue scheduling 3 subjects per week to reach our goal of collecting data on 400 ill and well veterans by the end of our 5-year recruitment period.

RESULTS

Thus far, 150 subjects have data for analysis. Subjects are categorized as GW Veterans (healthy controls), GW Illness (those who meet CDC criteria), or intermediate. The following table summarizes various demographic variables. "CAPS current" represents the mean score of the frequency and severity of the Clinician Administered PTSD Scale on a scale from 0-4 for 17 symptoms of post-traumatic stress. "Current drinking" represents the mean number of alcoholic drinks consumed monthly. "GWI severity" represents the mean score of Gulf War Illness as measured by a medical practitioner's clinical judgment. When compared to demographic data compiled for the previous year's annual report, the three groups are more closely matched with respect to age and education level.

Summary Variables

	GW Veteran	Intermediate	GW Illness
N	54	57	39
Age	42.11 ± 9.0	43.93 ± 11.3	41.31 ± 8.9
Education	14.73 ± 2.2	14.24 ± 2.2	14.27 ± 1.9
CAPS Current	9.60 ± 17.2	24.48 ± 26.2	32.19 ± 28.6
Current Drinking	12.90 ± 19.1	27.71 ± 86.7	8.55 ± 15.2
GWI Severity	1.75 ± 0.5	1.82 ± 0.9	2.82 ± 1.0

Spectral Data Analysis

NAA is a measure of neuronal density or integrity and can be measured as absolute NAA or a ratio to other metabolites. Tables 1 and 2 present preliminary spectral

data. These analyses have not controlled for the effects of PTSD, depression, and alcohol consumption.

Table 1: Mean Absolute NAA

	GW Veteran	Intermediate	GW Illness
Left BG	65.59 ± 11.0 n=27	64.24 ± 10.9 n=32	64.66 ± 11.0 n=27
Right BG	60.20 ± 9.0 n=31	60.05 ± 8.2 n=29	60.20 ± 9.0 n=25
Pons	36.58 ± 22.4 n=20	28.89 ± 6.2 n=30	30.24 ± 7.5 n=23

Table 2: Mean NAA/Cr

	GW Veteran	Intermediate	GW Illness
Left BG	1.66 ± 0.16 n=27	1.61 ± 0.16 n=32	1.68 ± 0.14 n=27
Right BG	1.66 ± 0.23 n=31	1.60 ± 0.15 n=29	1.61 ± 0.20 n=25
Pons	2.12 ± 0.38 n=20	2.15 ± 0.38 n=30	2.07 ± 0.68 n=23

Neurocognitive Tests

Haley et al. reported global intellectual and neurocognitive dysfunction among ill veterans when compared to control veterans. This study administered tests similar to those used by Haley in an attempt to replicate his findings. The following table summarizes the preliminary data for our neurocognitive battery. Heavy drinkers and subjects who failed a valid test of memory function were removed from this analysis. Post-hoc tests were corrected for multiple comparisons. Future analyses will control for effects of PTSD, depression, and alcohol consumption.

Neurocognitive Tests

	GW Veteran	Intermediate	GW Illness
WAIS Vocabulary	45.57 ± 9.8 n=37	44.28 ± 9.4 n=40	47.24 ± 6.1 n=33
WAIS Similarities	24.51 ± 4.3 n=37	23.10 ± 5.3 n=40	23.85 ± 4.1 n=33
WAIS Information	20.03 ± 4.4 n=37	18.30 ± 4.3 n=40	19.28 ± 3.2 n=33
WAIS Comprehension	24.11 ± 3.9 n=37	23.12 ± 4.1 n=40	24.48 ± 3.5 n=33

Neurocognitive Tests (cont'd)

	GW Veteran	Intermediate	GW Illness
WAIS Arithmetic	14.15 ± 4.4 n=41	13.52 ± 3.7 n=46	14.11 ± 3.4 n=37
WAIS Picture Completion	19.73 ± 4.2 n=41	19.65 ± 3.5 n=46	20.16 ± 3.2 n=37
WAIS Block Design	44.12 ± 13.2 n=41	41.87 ± 13.2 n=46	41.84 ± 9.7 n=37
WAIS Digit Symbol	71.85 ± 18.0 n=41	66.17 ± 13.8 n=46	69.51 ± 12.7 n=37
WAIS Digit Span	17.68 ± 5.4 n=41	16.39 ± 3.7† n=46	18.56 ± 3.7† n=36
Grip Dominant	38.76 ± 14.3 n=40	33.52 ± 15.2 n=45	35.72 ± 14.0 n=37
Grip Nondominant	37.16 ± 15.1 n=40	31.75 ± 14.0 n=46	33.78 ± 14.1 n=37
Pegboard Dominant	71.12 ± 15.7 n=41	71.20 ± 12.2 n=46	70.81 ± 13.0 n=37
Pegboard Nondominant	76.78 ± 17.3 n=41	77.35 ± 14.8 n=46	72.92 ± 14.7 n=37
WRAT Reading	48.93 ± 5.0 n=41	48.13 ± 5.7 n=46	48.65 ± 3.8 n=37
WRAT Spelling	41.49 ± 6.3 n=41	40.28 ± 5.8 n=46	41.95 ± 6.6 n=37
COWAT FAS	40.51 ± 10.7 n=41	39.24 ± 9.7 n=46	42.65 ± 8.5 n=37
COWAT Animals	22.28 ± 5.4†† n=40	20.31 ± 5.6 n=45	19.69 ± 4.4†† n=36
BVMT Immediate	24.31 ± 5.7 n=35	22.62 ± 6.3 n=40	23.72 ± 6.7 n=32
BVMT Delay	9.49 ± 2.3 n=35	9.00 ± 2.3 n=40	9.09 ± 2.3 n=32
Trails A	29.26 ± 12.7 n=41	29.58 ± 11.4 n=45	30.84 ± 11.7 n=37
Trails B	61.61 ± 29.6 n=41	67.80 ± 30.2 n=46	61.27 ± 18.3 n=37
Short Categories	29.00 ± 16.4 n=41	29.48 ± 15.6 n=46	25.59 ± 11.0 n=36
WMS Logical Memory Immediate	44.59 ± 10.5* n=41	39.37 ± 9.3* n=46	42.76 ± 11.0 n=37
WMS Logical Memory Delay	27.93 ± 7.7** n=41	22.15 ± 6.7** n=46	25.24 ± 7.8 n=37
CVLT Immediate	6.44 ± 2.0 n=39	5.98 ± 1.6 n=44	6.69 ± 2.1 n=29
CVLT Short Delay	12.31 ± 3.2*** n=39	10.32 ± 3.2*** n=44	10.83 ± 3.6 n=29
CVLT Long Delay	12.31 ± 3.1**** n=39	10.25 ± 3.6**** n=44	11.03 ± 3.4 n=29

†p=.08, Intermediate < GWI
††p=.08, GWI < GWV
*p=.02, Intermediate < GWV
**p=.002, Intermediate < GWV
***p=.02, Intermediate < GWV
****p=.02, Intermediate < GWV

Paraoxonase/Arylesterase Analysis

Paraoxonase/arylesterase 1 (PON1) status was ascertained for 107 individuals. Of these, 98 have had their CDC status determined. As expected, a plot of the rates of diazoxon hydrolysis vs. paraoxon hydrolysis divided the population into three distinct groups: individuals functionally homozygous for $PON1_{192Q}$ or $PON1_{192R}$ and heterozygotes. The functional enzyme analysis provides both an accurate inference of the amino acid present at position 192, which determines catalytic efficiency for the hydrolysis of some substrates, and the level of plasma PON1. Both of these parameters are important in determining an individual's sensitivity or resistance to a given exposure. The $PON1_{192Q}$ isoform has a higher diazoxonase activity level (a higher rate of hydrolysis of diazoxon, sarin, and soman), and the $PON1_{192R}$ isoform has a higher paraoxonase activity level (a higher rate of hydrolysis of paraoxon and chlorpyrifos oxon). Haley et al. reported that veterans homozygous for $PON1_{192Q}$ were less likely to have neurologic symptom complexes than those possessing the $PON1_{192R}$ allele. The preliminary rates of hydrolysis for each group are summarized below.

Rates of Hydrolysis

	GW Veteran	Intermediate	GW Illness
Paraoxonase	1009 ± 636 n=31	866 ± 505 n=39	1063 ± 742 n=28
Diazoxonase	9147 ± 2412 n=31	9254 ± 3436 n=39	9673 ± 3633 n=28

KEY RESEARCH ACCOMPLISHMENTS

- New staff hired and trained.
- Study manuals and protocols maintained.
- UCSF, VA, and DOD IRB approvals maintained.
- Subject recruitment letters mailed out: 2319
- Subjects requesting informational packet: 488
- Subjects entered into study: 176
- Continued preliminary analysis of data.

REPORTABLE OUTCOMES

- No publications at this early stage of the project.
- VA Headquarters in Washington, DC Meeting on Magnetic Resonance and Spectroscopy of Human Brain in Gulf War Illness. May 26th, 2003.
- NIH MR and Spectroscopy of Human Brain in Gulf War Illness Conference. Talk entitled "Magnetic Resonance and Spectroscopy of Human Brain in Gulf War Illness." February 19th, 2002.

CONCLUSIONS

After 2.5 years of data collection, the three study groups are well matched with respect to age and education level. The preliminary spectral data suggests there are no differences between groups in the basal ganglia bilaterally or the pons. The preliminary neurocognitive test data indicate possible group differences on measures of memory function. The preliminary PON1 data analysis shows no differences between groups with respect to paraoxonase and diazoxonase activity. However, thus far, none of our data has been corrected for the confounds of PTSD, depression, or alcohol consumption.

In summary, the results clearly show that we are able to recruit, study, and obtain usable data to meet the aims of this study. Although at this point in time, our data do not seem to replicate the findings of Haley et al, we do not have sufficient statistical power to derive any reliable conclusions. Further work is required and we expect that the full 5 yrs of funding will be needed to obtain data which support robust conclusions.